Public Health Reports

VOLUME 64 MAY 13, 1949 NUMBER 19

IN THIS ISSUE

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Public Health Reports

Vol. 64 • MAY 13, 1949 • *No. 19*

Pertussis and Aureomycin

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Pertussis is a universally prevalent disease which has been resistant to all methods of treatment that appear suitable for general use in the home. Aureomycin is a relatively nontoxic antibiotic which can be administered orally. It is a yellow crystalline substance obtained from the mold *Streptomyces aureofaciens* described by Duggar (*1*). Harned et al. (*2*) have described the pharmacology of aureomycin. Others (*3*) have described *in vitro, in vivo,* and clinical trials of aureomycin in a variety of specific infections, but not in pertussis. Since pertussis has been a major concern of two of the authors for many years, such studies were undertaken at the National Institutes of Health in December 1948.

The main objective of these studies was to determine whether aureomycin is of value in the treatment of experimental infection of mice with *Hemophilus pertussis* and, if of value, to initiate a clinical trial. The laboratory experiments were to determine whether aureomycin would protect mice against the effects of intracerebral infection induced with *H. pertussis,* and, if so, to obtain a general idea of the optimum treatment regime. Mice do not naturally acquire pertussis, and the results of aureomycin treatment of mice following a highly artificial intracerebral infection can only serve as a general guide for studies in the treatment of the respiratory infection in humans. The early favorable results of the laboratory experiments justified a clinical trial which was promptly undertaken. To date, 20 human cases have been treated in their homes with aureomycin, and the apparent beneficial effect prompts the inclusion of preliminary clinical findings in this report.

Laboratory Methods and Procedures

Equal numbers of male and female white mice from a closely inbred strain, weighing 15 to 18 gm., were distributed in groups of 10. They were infected by intracerebral inoculation of 0.03 ml. of a sus-

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pension of an 18- to 24-hour culture of *H. pertussis*, strain No. 7 (Kendrick 18323), in a solution of casein peptone. The technique of infection and the titration of the culture were the same as employed by the National Institutes of Health in the evaluation of antigenicity of pertussis vaccines (*4*). After infection the mice were observed daily, and usually at more frequent intervals, for 28 or 35 days. Cultures were made from the brains of all the mice, including those dying during the observation period and those killed at the termination of the experiment. The results were recorded as positive or negative depending upon whether *H. pertussis* was present or absent, and as unknown if there was overgrowth by other bacteria. Bordet–Gengou medium containing 17-percent rabbit blood was used for all cultural work.

Five laboratory experiments were made, and in each there was included a titration of the culture in 30 or more normal mice in order to calculate the LD_{50} of the controls. The mice treated with aureomycin received infecting doses varying from 2,000 to 200,000 organisms representing an LD_{50} from 1 to 700. For treatment, the aureomycin hydrochloride [1] was dissolved in neutral phosphate buffer immediately prior to use. It was injected subcutaneously in 0.25-ml. amounts when the single dose was 2.5 mg. or less, and in 0.5- or 1.0-ml. amounts when the single dose was 5 or 10 mg., respectively. The total amount of treatment was varied from 0.078 to 32.0 mg. of aureomycin. The interval between injection of the culture and beginning of treatment was varied from 6 to 96 hours. The frequency of treatment was varied from one to four times a day at equal time intervals, and the duration of treatment was varied from a single dose to 8 days of treatment.

All of these numerous variables were considered essential to obtain a general idea of the optimum treatment regime. In various combinations they represent hundreds of treatment regimes, the detailed analysis of which goes far beyond the stated objectives of the laboratory experiments. Since the mice were uniformly treated in groups of 10, it is permissible to combine these groups in logical order whenever the difference in the protection resulting from treatment in each of the small groups was not significant. Such combinations simplify the presentation of results. An example of the method used in combining these small groups is included.

Results

In the pilot experiment, 30 mice were inoculated intracerebrally with 2,000 organisms which represented approximately 3 LD_{50} (determined by titration of the culture in 30 additional mice). Twenty

[1] Contributed by Lederle Laboratories Division, American Cyanamid Co., through courtesy of Dr. B. W. Carey, director.

mice were treated with a total of 10 mg. of aureomycin divided into 10 doses given at 12-hour intervals beginning 6 hours after infection; the other 10 remained untreated. Only 1 of the 20 treated mice died (this occurred on the 17th day), while 7 of the 10 untreated mice died within 2 weeks. Of the 19 treated survivors, 9 were killed on the 21st day and the remaining 10 were killed on the 28th day together with the 3 untreated mice. The brain cultures of the 22 survivors were negative.

In the second experiment, 150 mice were used. The mice were inoculated intracerebrally in groups of 50 with 2,000, 20,000, and 200,000 organisms which represented approximately 1, 10, and 100 LD_{50}, respectively. As in experiment 1, treatment was started 6 hours after infection and a total of 10 mg. of aureomycin was given each mouse, either in a single dose or divided into 2, 5, or 10 doses. Only 17 of the 120 treated mice died, while 21 of the 30 controls which received corresponding doses of the culture died. The average time of death in the treated mice was 20 days and in the controls 8.7 days after infection. All surviving mice were killed on the 35th day and their brain cultures were negative, except that of 1 control which was positive.

In the third experiment, the mice were inoculated intracerebrally with 100,000 organisms which represented approximately 200 LD_{50}. The virulence of the culture used in this experiment had been increased by one mouse brain passage. The treated mice were given a single dose of aureomycin, but the amount and the interval between infection and treatment was varied. The results are summarized in table 1. No protection was evident when the single treatment dose was given 72 or 96 hours after infection. It is noteworthy that these mice were all obviously ill and many were paralyzed at time of treatment. Mice receiving a single small dose of 0.313 mg. or less showed no evidence of protection except possibly a slight delay in time of death. On the other hand, mice given a single dose 24 or 48 hours after infection showed a definite delay in time of death; those treated at 48 hours also showed a substantial proportion of survivors.

The essential findings of experiment 4 are given in table 2. The infecting dose was again 100,000 organisms from the same culture used in experiment 3. The infecting dose represented approximately 700 LD_{50}. Varying amounts of aureomycin were given in 1 to 8 doses beginning 24, 48, or 72 hours after infection. Table 2 shows again that treatment begun 72 hours after infection (group *b*) gave no evidence of protection. The smaller doses of the drug given 24 hours after infection (group *c*) gave a definite delay in time of death. Mice given a total of 5 or 10 mg. of aureomycin in divided doses starting 24 or 48 hours after infection (groups *d* and *e*) showed not only a delay in time of death but also a substantial proportion of survivors. Mice

Table 1. *Experiment 3—Mortality and cultural results in mice treated with a single dose of aureomycin*

[Infection dose = 100,000 organisms equivalent to 200 LD_{50}]

Group	Interval between infection and treatment in hours	Total amount drug in mg.	Total number of mice	Number of mice dying during observation period								Number of mice surviving				*H. pertussis* culture total	
				Total	Week of death				Culture			Total	Culture				
					1	2	3	4	+	−	Unk.		+	−	Unk.	+	−
a	0	0	20	20	19	1			14	0	6	0			0	14	0
b	72, 96	10–0.625	70	67	59	7	1	0	52	2	13	3	0	3	0	52	5
c	24, 48, 72	0.3–0.08	60	57	39	17	1	0	48	0	9	3	1	2	0	49	2
d	24	10–1.25	37	34	0	20	14	0	28	0	6	3	0	2	1	28	2
e	48	10–1.25	40	19	3	5	7	4	15	2	2	21	0	21	0	15	23
PERCENTAGES																	
a, *b*, *c* [1]			100	96	78	17	1	0	76	1	19	4	1	3	0	77	5
d	24 hours		100	92	0	54	38	0	76	0	16	8	0	5	3	76	5
e	48 hours		100	48	8	13	18	10	38	5	5	52	0	52	0	38	58

[1] Untreated, late treatment, small doses.

EXAMPLE OF GROUP COMBINATIONS

The following basic data are presented to exemplify the method of combining various groups of 10 mice into larger groups for summary in tables 1 and 2. The mice below from experiment 3 (table 1) received only one dose of aureomycin 48 hours after infection.

Aureomycin mg./mouse	Number of mice	Day of death following infection									
None (controls)	10	5	5	5	5	5	6	6	6	6	10
0.078	10	5	6	6	6	7	7	7	7	7	8
0.313	10	7	7	8	8	9	9	9	11	S	S
1.25	10	11	12	12	13	20	S	S	S	S	S
2.50	10	15	16	[1]18	25	S	S	S	S	S	S
5.0	10	13	16	16	16	S	S	S	S	S	S
10.0	10	4	7	7	22	23	[1]28	S	S	S	S

[1] These and all survivors (S) had negative brain cultures for *H. pertussis*.

It is apparent that there was no important difference in protection between the four groups of mice receiving a single dose of aureomycin ranging from 1.25 to 10.0 mg. Forty mice receiving these doses are tabulated as group *e* in table 1. The group of mice receiving 0.313 mg. showed the same pattern of death as the group receiving 0.078 mg., and this pattern was like that of other groups treated with the same dosages 24 or 72 hours after infection. These mice are included in group *c* in table 1.

receiving 4 doses of drug beginning 48 hours after infection fared as well as and perhaps better, in view of cultural results, than those receiving 8 doses of the same total amount (either 5.0 or 10.0 mg.) starting 24 hours after infection.

In the fifth experiment the infecting dose was 20,000 bacteria, representing approximately 200 LD_{50}. Treatment was begun 6 or 48 hours after infection, and 2.0, 0.5, or 0.125 mg. of aureomycin was given

Table 2. *Experiment 4—Mortality and cultural results in mice treated with various doses of aureomycin*

[Infection dose = 100,000 organisms equivalent to 700 LD_{50}]

Group	Interval between infection and treatment in hours	Number of doses [1]	Total amount drug in mg.	Total number of mice	Number of mice dying during observation period								Number of mice surviving				*H. pertussis* culture total	
					Total	Week of death				Culture			Total	Culture				
						1	2	3	4	+	−	Unk.		+	−	Unk.	+	−
a	0	0	0	30	30	27	3	---	---	30	0	0	0	---	---	---	30	0
b	72	1–8	20–2.5	70	70	62	6	2	---	67	1	2	0	---	---	---	67	1
c	24	8	2.5, 1, 0.5	29	25	0	23	0	2	24	1	0	4	2	2	0	26	3
d	24	8	10, 5	20	9	0	5	3	1	9	0	0	11	1	10	0	10	10
e	48	4	10, 5	20	9	2	1	2	4	4	2	3	11	0	11	0	4	13
PERCENTAGES																		
a, *b* (Untreated and late treatment)				100	100	89	9	2	---	97	1	2	0	---	---	---	97	1
c (Small doses)				100	86	0	79	0	7	83	3	0	14	7	7	0	90	10
d, *e* (Moderate doses)				100	45	5	15	12	12	32	5	8	55	2	52	0	35	58

[1] Interval between doses = 12 hours.

per dose with 6-, 12-, or 24-hour intervals between doses for periods of 2, 4, or 8 days. In this, as in previous experiments, positive cultures were obtained almost uniformly from mice dying during the observation period, particularly from those dying during the first 3 weeks, and negative cultures were the rule from mice surviving 28 days. The groups of mice having low mortality had a delayed time of death as compared with those having high mortality. Thus, table 3 shows only the number of mice surviving 28 days in each separate group of 10 treated mice. These groups are arranged according to the combinations of treatment variables, and the total amount of treatment given each group is indicated at the bottom of the table by dotted connecting lines. By inspection it is seen that the findings of previous experiments were confirmed. It is obvious that treatment for 8 days gave better protection than treatment for 4 or 2 days, and that treatment with the larger doses gave complete protection, whereas the smaller doses did not, except when treatment was begun 48 hours after infection.

In experiment 5 the amount of aureomycin per dose that would protect 50 percent of the mice on the different treatment schedules was calculated by the Wilson-Worcester method (*5*). Table 4 shows the results of these computations. It shows that decreasing dosages were required to protect 50 percent of the mice either as the duration of treatment was prolonged or as the number of doses per day was increased. It is possible that this result may be due in whole or in part to the total dosage given. To investigate this, groups of mice receiving the same total dose were paired when the two groups differed

Table 3. *Experiment 5—Number of mice surviving 28 days in each group of 10 mice treated as specified*

[Infection dose = 20,000 organisms equivalent to 200 LD_{50}]

Interval infection to treatment (hours)	Duration of treatment (days)	Milligrams of aureomycin per dose										
		2.0			0.5			0.125			0.063	
		Doses/Day			Doses/Day			Doses/Day			Doses/Day	
		4	2	1	4	2	1	4	2	1	4	
48	8				10	7	4	8			9	
6	8	*6	10	10	9	8	1	7	4	3		
6	4	*8	10	2	4	0	1	2	0	0		
6	2	9	5	0	1	0	0	1	0	0		
Total No. Mg.		16+	8	4	2		1	0.5	0.25			

*4 and 2 mice in these 2 groups died on third to fifth day apparently from toxicity of drug as the cultures were negative. Treatment of these 2 groups was discontinued on the fourth day.

Table 4. *Experiment 5—The calculated number of milligrams of aureomycin required per dose to protect 50 percent of the mice*

Duration [1] of treatment (days)	Number of doses per day		
	4 (q 6 hr.)	2 (q 12 hr.)	1 (q 24 hr.)
8	0.057 (0.023–0.142) [2]	0.17 (0.12–0.24)	0.58 (0.41–0.83).
4	0.43 (0.32–0.58)	0.54 (0.42–0.68)	>2.0.
2	0.88 (0.67–1.17)	2.0 (1.5–2.7)	No protection.

[1] Treatment began 6 hours after infection.
[2] The figures in parentheses represent the limits of one standard deviation expressed in number of mg.

only as to whether they received treatment once or a total of two or four times a day. In six of seven such pairs there were more survivors in the groups receiving treatment two or four times a day than in those treated once a day, but in no one pair was the difference significant. In total, however, there were only 18 survivors out of 70 mice receiving one treatment a day, whereas there were 32 survivors among the 70 mice receiving multiple doses per day. This was significant when account was taken of the different total dosages and different durations of treatment. Thus, in general, the smaller doses given two or four times daily over the longer period of time were more beneficial than larger doses given once daily over the shorter period of time.

Summary of Experimental Results

Throughout these experiments it was observed that treatment beginning 48 hours after infection was more effective than treatment begun 6, 24, 72, or 96 hours after injection of the culture. This phenomenon is the subject of further study. It was also observed that groups of mice receiving large doses of the antibiotic had a substantial proportion of survivors, but deaths occurred in two patterns—one early and one late. This is evident in tables 1, 2, and 3 and in the example to table 1. Not infrequently negative brain cultures were found among mice dying early, which was unusual for mice dying during the observation period. These deaths are attributed to combined toxicity of the infection and treatment. A single dose of 10.0 mg. of aureomycin apparently approached the toxic level in infected mice but was not toxic for noninfected mice. It is concluded from the laboratory data that aureomycin given subcutaneously to white mice in doses which did not apparently impair their health delayed and prevented deaths following intracerebral infection with *H. pertussis*. In general, a treatment regime of small doses given at frequent intervals over a period of several days was more effective than larger doses given singly or at infrequent intervals for a few days.

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Preliminary Clinical Findings

Clinical trial of aureomycin is under way in Norfolk, Va., where one of the authors has continued a study of pertussis for more than 10 years. The study has been described elsewhere (*6*) and provides detailed clinical records of the duration, frequency and intensity of cough, paroxysmal cough, whooping, and vomiting in many untreated cases of pertussis. The data have been collected with meticulous uniformity, and the untreated cases appear suitable for controls in a study of aureomycin treatment carried out in the same general population group by the same observers.

The Norfolk Health Department, local physicians, and visiting nurse associations were requested to report all early cases of pertussis so that they could be studied to evaluate aureomycin treatment. To date, 20 cases have been treated and observed long enough to indicate the results. All treated cases had definite paroxysmal cough with vomiting, and whooping in all but 4. These 4 were younger siblings of patients whose cases were characterized by whooping. In each case, nasal pharyngeal swabs were taken in the homes by Public Health Nurses Anne Hodges and Mary Miller on the two consecutive days prior to treatment. The swabs were cultured for isolation of *H. pertussis* by the Norfolk City Health Department laboratory under direction of A. D. Farmer. A positive bacteriological diagnosis was reported when a gram-negative organism was isolated that had the cultural appearance, morphology, and agglutination characteristics of *H. pertussis*. Cultures from the first 7 cases were negative, but the relatively large amounts of penicillin used in the culture media for these cases was later found to inhibit growth of *H. pertussis*. Of the next 13 cases, 9 yielded positive cultures and 4 negative cultures. Three of these with negative cultures had siblings who gave positive cultures and the remaining case with a negative culture was observed repeatedly by the nurses during typical paroxysms with whooping and vomiting. Thus, substantial evidence supports the opinion that all treated illnesses were pertussis.

The treatment schedule was designed for home use. The time of starting treatment varied from the second to the seventeenth day following onset of paroxysmal cough. In the first 8 cases an effort was made to give a total of 0.5 gm. of aureomycin per kilogram of body weight in divided doses over a period of 4 days. When additional laboratory data were available, the schedule was changed to give the same total dose over an 8-day period. It is noteworthy that the amount of drug retained by small children having a disease characterized by frequent vomiting can only approximate the scheduled dosage. The administration of the aureomycin had to be supervised by the nurses in many instances. The usual procedure consisted in putting

the contents of one or two 250-mg. capsules in a cup, mixing it with a teaspoonful of sweet cherry syrup, and immediately administering it orally. When vomiting occurred immediately, the procedure was repeated.

The age of the patients varied from 1 month to 6 years. The number of cases is too few to evaluate the influence of the numerous attributes, e. g., age, sex, etc., which might possibly affect the clinical course of the disease. These will be analyzed in a future report. Paroxysmal coughing often attended by whooping and vomiting—defined in a previous report (*6*)—is the only uniformly characteristic clinical manifestation of pertussis. Accordingly, the duration, frequency, and intensity of paroxysmal cough are the chief criteria for evaluating the effect of the therapeutic agent. In this study the mothers kept a daily record of the occurrence, frequency, and intensity of paroxysmal cough, whooping, and vomiting, and the nurses visited almost daily to check the reliability of the data and to make sure that it was recorded in a uniform manner.

Table 5 shows the duration of paroxysmal cough in the 20 treated cases and 380 untreated controls. The latter represent some of the nonvaccinated and nontreated cases of the study referred to above. It will be noted that the duration of paroxysmal coughing in the control cases occurring during 1938 to 1942 was essentially the same as in those occurring during 1942 to 1947. This confirms the uniformity with which the data have been collected over a long period of time and is good evidence of the adequacy of these cases for controls.

The duration of paroxysmal coughing was less than 30 days in only 21 percent, or 79 of the 380 untreated controls, whereas the duration was less than 30 days in 85 percent, or 17 of the 20 treated cases (table 5). The question arises as to whether this favorable result in the treated cases was due to the aureomycin treatment per se or to other unrecognized causes. Further study of a larger experience will be necessary to answer the question. However, the data show

Table 5. ***Duration of paroxysmal cough in treated and untreated cases of pertussis***

	Interval onset Pe to treatment (days)	Year of occurrence	Total cases	Days duration of paroxysmal cough			
				<20	20–29	30–39	40+
Untreated cases		1938–42	165	11	25	42	87
		1942–47	215	14	29	47	125
Total			380	25	54	89	212
Aureomycin treated cases	2–10	1949	7	7	0	0	0
	11–17	1949	13	0	10	2	1
Total			20	7	10	2	1
Expected number of treated cases if distributed like untreated cases			20	1	3	5	11

that among the treated cases the shortest duration of paroxysmal cough occurred in those treated early in the course of the disease. Thus, the duration of the clinical disease was directly related to time treatment was started, and the favorable result appears to be due to the aureomycin treatment. Whooping and vomiting are part of the characteristic paroxysmal cough of pertussis, and their duration was curtailed in a like manner.

The daily record of the frequency of paroxysms showed a prompt but gradual reduction in the number of paroxysms in practically all treated cases. This was directly related to the period of time treatment was administered. The mothers of nearly all treated children were unanimous in their opinion that the severity of the paroxysms was promptly modified. They particularly noted the early subsidence of night coughing and vomiting. Some noted loose stools and yellow urine in children retaining the larger doses of aureomycin.

Summary and Conclusion

Aureomycin hydrochloride given subcutaneously to mice in apparently nontoxic doses not only delayed the time of death but also prevented deaths due to prior intracerebral infection with *H. pertussis*. Small doses of the drug given at 12-hour intervals over a period of 8 days were more effective than large doses given over a short period of time.

Preliminary clinical trials in 20 cases of pertussis when compared with 380 untreated cases suggest that aureomycin given orally in apparently nontoxic doses shortened the clinical course of the disease. In only a few, particularly the cases treated early, was the clinical response to aureomycin considered dramatic in the sense that complete recovery immediately followed a few days of treatment. However, in practically all cases a prompt but gradual diminution in the frequency and intensity of paroxysms was observed. Some difficulty was encountered in making sure that specified amounts of the drug were retained by young children with pertussis. No untoward effects resulted from treatment.

Aureomycin hydrochloride is effective for treatment of *H. pertussis* infection in mice and looks promising for treatment of the human disease. Further clinical trials are necessary to establish its value for general treatment of clinical pertussis.

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Simple and Efficient Transport Method for Gonorrheal Specimens

By Lenore R. Peizer, B. A., Gustav I. Steffen, Ph. D. and Sarah Klein, B. A.*

The culture method is the most valuable aid in the diagnosis of gonorrhea and in the establishment of cure. The method of transportation of gonorrheal specimens from the source to the laboratory plays an important role in determining the accuracy of culture diagnosis. Even when the source is close enough so that the specimens reach the laboratory within 4 hours, the method of conveyance is important. Thus, specimens streaked on solid medium at the clinic and transported to the laboratory yield more accurate results than do those placed into a liquid transport medium (*1*). When specimens cannot reach the laboratory within 20 to 24 hours, accurate culture diagnosis becomes even more difficult.

Various methods of transportation of gonorrheal specimens have been studied in our laboratory for a number of years and the method described in this paper appears to be simpler and more practical than those generally employed in localities where direct messenger service is not available.

The following simple medium is effective during a transport period of 24 hours:

To one liter of distilled water add:

5 grams corn starch.	3 grams NaCl.
10 grams Bacto peptone.	10 grams Bacto agar.

Stir well and heat in the Arnold sterilizer to dissolve the ingredients. Distribute in convenient amounts and sterilize at 15 pounds pressure for 20 minutes in the autoclave. To each 30 ml. of the melted cooled agar base add the following sterile solutions under sterile conditions in the following order:

2.1 ml. 15% Na_2HPO_4. $12H_2O$.
0.2 ml. 10% cysteine monohydrochloride.
1.0 ml. 1% $CaCl_2$ (anhydrous).
5.0 ml. 20% bovine albumin "V" fraction in saline.[1]
1.0 ml. 0.08% gentian violet (powder) in distilled water.[2]

[1] Bovine albumin powder fraction from bovine plasma. Manufactured and packed by the Armour Laboratory.

[2] Gentian Violet U. S. P. Fisher Scientific Co., Pittsburgh, Pa.

Tube 2 ml. quantities of the medium into 4″ x 7/16″ I. D. test tubes. After the medium hardens most of the air in the tubes is replaced with gaseous CO_2 and the cotton plugs are replaced by sterile rubber stoppers.

*From the Bureau of Laboratories, New York City Department of Health.

Mailing outfits, each consisting of one or two carrying tubes containing sterile swab applicators in a mailing container, may easily be assembled. A slip with instructions and a case history slip may be included. The medium in the carrying tubes remains usable for at least 2 months. Carrying tubes containing medium should be kept in the dark. When kept for over a week, they should be stored in a refrigerator.

The largest possible quantity of suspected fresh discharge should be taken from the patient by means of a swab applicator. While holding the carrying tube vertically to reduce the loss of CO_2, the applicator, of the right length to fit into the tube, is stuck into the agar. The rubber stopper is replaced securely. When the specimen reaches the laboratory, the applicator swab is removed from the carrying tube and is used for inoculation of medium suitable for primary cultivation of the gonococcus.

This carrying medium is a solid medium prepared from dry ingredients, all of which are easily obtained. It does not support primarily gonococcus growth under optimal conditions.

Experimental

Control and transport specimens were taken simultaneously from 125 female patients with acute gonorrhea at the Fort Greene Clinic in Brooklyn, N. Y. The control specimens were streaked on the Peizer-Steffen (*2*) medium in the clinic and the plates were incubated within 6 hours after the seeding. The transport specimens remained in the carrying tubes at about 20°–26° C. for 24 hours. They were then used to inoculate plates which were incubated for 40 hours at 36° C. in 10 percent CO_2. Corresponding control and transport cultures were then compared.

The results as seen in table 1 indicate that there was generally a decrease in the number of gonococcus colonies in cultures from the

Table 1

Control cultures incubated within 6 hours		Transport cultures incubation delayed 24 hours	
Approximate number gonococcus colonies	Number specimens	Approximate number gonococcus colonies	Number specimens
300+	14	300+	10
200–300	30	200–300	22
100–200	46	100–200	33
25–100	21	25–100	32
1–25	12	1–25	19
0	2	0	9
Total	125		125

transport medium. Nine specimens, about 7 percent, became negative during the transport period. On the other hand, two specimens, about 1.5 percent, which were positive in the transport medium were negative in the direct culture group.

Nile Blue A and gentian violet were tested as inhibitors of contaminants. It was found that the gonococcus tolerates Nile Blue A in the transport medium up to 10 times the amount it safely tolerates in the medium used for primary cultures. When 12.5 times this amount was used, however, definite inhibition of gonococci in the transport specimens occurred. It was found, on the other hand, that Nile Blue A, though effective in medium used for primary cultures, did not sufficiently inhibit many of the contaminants in the transport medium even where the maximum amount was employed.

It is known that when gentian violet is used in effective amounts in media employed for primary gonococcus growth, it completely inhibits about 12 percent of known positive cultures. However, 0.002 percent of gentian violet incorporated in this carrying medium effectively checked the growth of most concomitant organisms in the specimens and did not seem to be toxic for the gonococcus during the transport time of 24 hours.

Three specimens were taken simultaneously from each of 90 positive female patients. One specimen was used for streaking a control plate; another was placed into a carrying tube containing transport medium with 0.0024 percent Nile Blue A; the third specimen was placed into a carrying tube of transport medium with 0.002 percent of gentian violet. The control plates were incubated within 6 hours. The transport specimens were taken out of the tubes after 24 hours at about 20°–26° C. and were used to inoculate Peizer-Steffen agar plates. Transport cultures were compared, after incubation with their respective controls and with each other.

Table 2 shows results obtained when 0.002 percent of gentian violet was used and when 0.0024 percent Nile Blue A (10 times the amount

Table 2

90 positive control specimens incubated within 6 hours (Peizer-Steffen agar)		90 positive specimens incubation delayed 24 hours							
		Transport medium with 0.0024 percent Nile Blue A				Transport medium with 0.002 percent gentian violet			
		Number negative		Number positive		Number negative		Number positive	
Contaminated [1]	Apparently pure	Sterile	Overgrown	Contaminated	Apparently pure	Sterile	Overgrown	Contaminated	Apparently pure
58	32	3	10	56	21	3	2	51	34

[1] Positive gonococcus cultures were called contaminated when contaminating colonies were evident on gross examination and were called apparently pure when no contaminating colonies were seen on gross examination.

used in primary culture plates) was incorporated in the transport medium. As seen in the table, 13 cultures or about 14 percent were lost in the carrying medium with Nile Blue A, while 5 cultures or about 5.5 percent were lost in the carrying medium with gentian violet. The loss of most of the positives in the Nile Blue A tubes was due to overgrowth by contaminants.

It is believed by some (*3*, *4*) that CO_2 helps preserve gonococci in specimens during transportation. The effect of CO_2 on positive specimens during transportation in this medium was determined by omitting it from the carrying tubes of one set of specimens and including it in another set taken simultaneously from each of 79 patients with acute gonorrhea.

Results shown below indicate no significant difference with this particular transport medium. In previous tests, the McClure and Miller (*5*) method of transportation yielded 98 percent of positives when CO_2 was used and 94 percent when omitted; the Usher and Stein method yielded 89 percent with CO_2 and 79 percent without it.

	Number	*Percent*
Control cultures streaked on Peizer-Steffen medium, incubation within 6 hours	79	100
Transport specimens, incubation delayed 24 hours:		
With CO_2	75	94
Without CO_2	73	92

Discussion

During this study it became apparent that since gonococcus does not multiply at temperatures prevalent during transportation of specimens, the various growth stimulating substances usually added to transport medium serve no practical purpose. If there is any effect, it is detrimental since the enriched medium stimulates luxuriant growth of the usual contaminants in the specimens, which grow well at transport temperatures. Many positive gonococcus cultures are lost because of overgrowth by contaminants.

Substances needed in a transportation medium are those which serve only to preserve the gonococci in positive specimens and those which inhibit multiplication of the usual concomitant organisms found in gonorrheal specimens. The transport medium described here does not support the growth of gonococci. It does, however, conserve most of the organisms in positive gonorrheal specimens.

It is important in preparing the medium, to add the ingredients to the melted, cooled agar in the order given in the formula, to prevent the formation of a precipitate which tends to inactivate the cysteine. When cysteine is used in medium containing plasma, it seems to be most effective when added to the cooled, melted agar before the enrichment. The amount of cysteine giving the best results was found to be 0.02 to 0.05 percent. Larger amounts tend to inhibit the growth of gonococci.

Summary

1. A simple carrying medium for transportation of gonorrheal specimens is described. The medium is prepared from easily obtainable ingredients and may be stored in the refrigerator for at least 8 weeks. It does not support primary growth of gonococci. It is effective as a transport medium during 24 hours of delay in inoculation and incubation of isolation culture plates.

2. It was found that in this medium 0.002 percent of gentian violet is more effective as an inhibitor of contaminating organisms than 0.0024 percent of Nile Blue A. This amount of Nile Blue A is 10 times that used effectively in medium for primary gonococcus growth.

3. The addition of CO_2 to the transport medium tube had no significant effect.

4. Mailing outfits may be easily assembled.

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Diarrheal Disease Control Studies

II. Conical Net for Collecting Flies

By Paul P. Maier and Richard P. Dow*

It is sometimes necessary to collect very large numbers of muscoid flies for laboratory studies. The success of these investigations may depend on uniform freshness of specimens, wide representation of the local fauna, freedom from artificial contamination, and other considerations such as natural occurrence in a specific place (not attracted by bait from a distance).

The customary baited traps are not satisfactory for several reasons. First, they are large and clumsy to handle. Second, it may be difficult to procure them in the required numbers on short notice. Third, the baits to be used as attractants must be selected with regard not only to sterility, but also their ability to compete with other fly-attracting materials in the same surroundings. Fourth, since a trap must usually be exposed for a number of hours, the flies caught are not in equally fresh condition, and if only live material is desired, it is difficult to separate living from dead specimens. Finally, traps are frequently upset or stolen.

The conventional insect net is likewise impractical when large collections must be made. Its principal shortcoming lies in the fact that when swept over any sizable concentration of flies, it disturbs more individuals than it catches, and much time is lost in waiting for the scattered flies to return. Other objections are the difficulties met in transferring the specimens to containers suitable both for transportation to the laboratory and for handling the collections upon arrival.

A new type of conical net obviates all of these difficulties. Its operation takes advantage of the tendency of muscoid flies to fly toward light when the illumination of their environment is suddenly reduced. It was found that a cone of wire screening could be moved over an area attractive to flies and lowered to the ground without dispersing more than a few individuals. By placing a cylindrical cage of wire screening over a small hole in the top of the cone, and then rapidly covering the rest of the cone with a cape of heavy cloth (fig. 1), it was possible to concentrate all the trapped flies in the cage within a few seconds.

The fly cone is 30 inches in diameter at the base, but so light (2½ pounds) that it can be handled very easily by the small ring at

Part I, Effect of Fly Control in a High Morbidity Area, was published in Public Health Reports 63: 1319-1334 (1948).

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Figure 1. Spreading cape around fly cone.

Figure 2. Operator ready to close collecting cage.

the top. In addition to being lowered over an attractive surface, the cone can be held at an angle and used to sweep flies out of weeds or toward more level ground. When an operator has acquired the necessary skill, it can even be employed to catch flies flushed from a garbage can or barrel.

The cloth cape of a dark material, such as blue denim, is cut to fit the cone, but is large enough to extend 6 inches beyond the base. This is important because flies will escape near the ground if light enters under the net. In fact, it is always wise to close the cape first at the bottom.

The collecting cage, which fits on the ring at the top of the cone, is set in place just after the flies have been trapped. It can be made of the same wire screening as the cone, but 20-mesh is preferable in order to prevent the escape of smaller flies. The lid, which is slipped over the end of the cage just as it is lifted from the cone, is held in place by clamps made of metal stripping (fig. 2). In a dry or excessively hot climate, the cages should be kept in a cool, or at least humid, container until the flies can be brought to the laboratory where they are anesthetized and sorted.

The apparatus is constructed as follows:

The cylindrical cages (fig. 3A) of 20-mesh screening sewed with wire (*a*) are soldered to rings ⅝-inch wide (*b*), which are cut from No. 3 cans and flared to fit unused can lids (also size 3) as supplied to canneries. A cone of 18-mesh galvanized wire screening, 30 inches in diameter at base, is fitted over a framework of 9-gage galvanized wire, 17 inches high (fig. 3C). The upper ends of the four supporting rods (fig. 3B, *c*) are bent to fit under a wire ring of 3¼ inches inside diameter (*d*) and are soldered to the ring (fig. 3D). The cone of screening (*e*) is next soldered to the top of the framework, and after the outer margin of the screening has been bound with adhesive tape, it is rolled over the wire ring of the base and sewed in place with copper wire, which is also used to close the seam. The screening is left free from the supporting rods. A strip of banding steel ⅜-inch wide (*f*) is riveted to form a ring 3¾ inches in diameter and soldered firmly to the top of the cone. Finally, a very flat cone of screening (*g*), with a hole at the apex about ¾-inch in diameter, is soldered to the inner surface of the ring assembly.

For making large collections of common calypterate and acalypterate flies for medical studies, the fly cone has more than demonstrated its efficiency. Since first used, it has been found to have other applications. One is to collect flies following their exposure to space sprays in order to observe the lasting effect of the insecticide. This permits comparative studies of both equipment and insecticides without the disadvantage of having to expose test specimens in

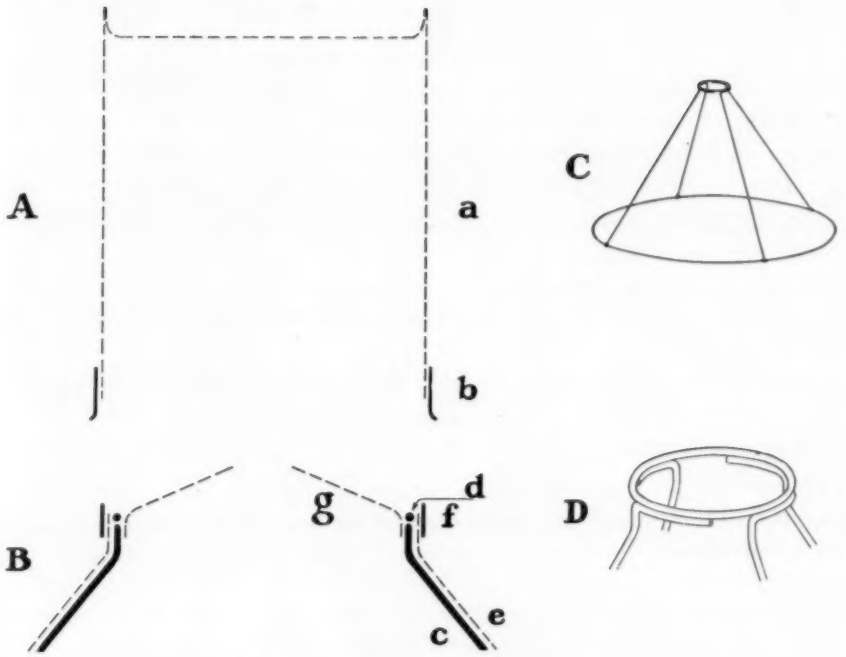

Figure 3.—Construction of fly cone.

A. Diagrammatic section of collecting cage.
B. Diagrammatic section of top of fly cone.
C. Diagram of framework.
D. Detail of supporting rods.

cages with screening which interferes with the dispersion of the spray. A second use is to study the various species of flies attracted to particular baits, such as various foods and excrement of different animals. A third is the collection of wild flies which have been allowed to feed on sterile food. A fourth application, for which a larger pyramidal model has been built, is to evaluate the method of measuring fly densities which was developed by H. I. Scudder.[1] To study the fly counts obtained by the grill method, the net is lowered over the grill as soon as the reading is complete, and the resulting collection of flies is identified for comparison with the species break-down of the grill count. Other uses for the fly cone are likely to be found as the need develops.

[1] Scudder, H. I.: A new technique for sampling the density of housefly populations. Pub. Health Rep. 62: 681–686, plate 1 (1947).

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July–December 1948

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*The Journal of Venereal Disease Information (monthly) July–December, vol. 29, Nos. 7 to 12, pages 193 to 403. 10 cents a copy. Subscription price 75 cents a year.

*Journal of the National Cancer Institute (bimonthly) August–December, vol. 9, Nos. 1 to 3, pages 1 to 260. 40 cents a copy. Subscription price $2 a year.

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INCIDENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED APRIL 23, 1949

For the current week a total of 29,165 cases of measles was reported, as compared with 27,750 last week and a 5-year (1944–48) median of 27,438 (reported for the corresponding week last year). An aggregate increase of 2,605 cases was reported in the Middle Atlantic, North Central, South Atlantic, East South Central, and Mountain areas. A combined decrease of 1,190 cases occurred in the New England, West South Central, and Pacific areas. The largest numbers (last week's figures in parentheses) were reported in the Middle Atlantic area, 7,381 (6,117), East North Central, 4,059 (3,928), South Atlantic 4,540 (3,778), and West South Central, 3,584 (4,305). The 5 States reporting more than 1,500 cases each (last week's figures in parentheses), are New York, 2,834 (2,475), New Jersey 2,213 (1,586), Pennsylvania 2,334 (2,056), Wisconsin 1,889 (1,984), and Texas 2,405 (3,013). To date this year 372,880 cases have been reported, as compared with a 5-year median of 278,171 (reported last year).

Of the total of 2,288 cases of influenza, as compared with 2,606 last week and a 5-year median of 1,691, only 2 States reported more than 195 cases—South Carolina 623 (last week 304), and Texas 931 (last week 1,009). The total to date is 63,323, 5-year median 180,632.

Of 45 cases of poliomyelitis reported, the same number as last week's total, Utah reported 8, Texas 7, California 5, Mississippi 4, and North Carolina 3, no other State reporting more than 2 cases. The total for the corresponding week last year was 39 and the 5-year median is 32. The total since March 19 (average date of seasonal low incidence) is 225 and the corresponding 5-year median 142.

During the week, 5 cases of smallpox were reported, 1 each in Missouri, South Dakota, Kentucky, Texas, and New Mexico; 4 cases of Rocky Mountain spotted fever, 1 each in Ohio, Maryland, Virginia, and Idaho. New York reported 1 case of anthrax.

During the week 9,521 deaths were recorded in 94 large cities in the United States, as compared with 9,232 last week, 9,226 and 9,513, respectively, for the corresponding weeks of 1948 and 1947, and a 3-year (1946–48) median of 9,502. The total for the year to date is 156,890, as compared with 160,837 for the corresponding period last year. Infant deaths totaled 638 for the week, last week 608, 3-year median 662. The cumulative figure is 10,553, same period last year 11,079.

Telegraphic case reports from State health officers for week ended April 23, 1949

[Leaders indicate that no cases were reported]

Division and State	Diphtheria	Encephalitis, infectious	Influenza	Measles	Meningitis, meningococcal	Pneumonia	Poliomyelitis	Rocky Mountain spotted fever	Scarlet fever	Smallpox	Tularemia	Typhoid and paratyphoid fever [d]	Whooping cough	Rabies in animals
NEW ENGLAND														
Maine	1		3	426	1	10			14				26	
New Hampshire			1	87		2			4				7	
Vermont				158					4			1	4	
Massachusetts	20	1		748	1				117			2	63	
Rhode Island				146		11			11			1	2	
Connecticut	15		3	1,418		53			37				11	
MIDDLE ATLANTIC														
New York	9	4	[b] 1	2,834	8	383			[c] 260			2	140	5
New Jersey	2		2	2,213		72	2		146				44	2
Pennsylvania	10	1	(b)	2,334	2		1		202		1	3	73	1
EAST NORTH CENTRAL														
Ohio	5		4	921	6	77		1	261			1	38	13
Indiana	8		1	211		16			43		1	2	19	21
Illinois	1		8	232	7	134	1		138		1	2	62	1
Michigan [a]	1		3	806	2	88			309		1	2	33	1
Wisconsin			39	1,889	2	6			45				29	
WEST NORTH CENTRAL														
Minnesota	1			197		6			32					
Iowa				67	1	4	2		22				5	9
Missouri	1		1	340	2	9	1		11	1		2	2	
North Dakota			17	45	1				2					
South Dakota				11	1				1	1				
Nebraska			2	161		1			11					
Kansas			12	1,126	1	26			7				14	
SOUTH ATLANTIC														
Delaware				67			1		9				2	
Maryland [a]	3		1	401	1	50		1	[c] 32			2	6	
District of Columbia			1	123		25			9				4	
Virginia			195	1,056	4	69		1	15			1	20	
West Virginia			17	115	1	9			20			2	15	
North Carolina	3			967	4				15					
South Carolina	7	2	623	612	3		3					1	27	
Georgia	2		13	230		175			9				12	

District of Columbia			195	1,056	4	69			20			2	15	
Virginia			17	115	1	9								
West Virginia														
North Carolina	3			967	4		3		15			1	27	
South Carolina	7	2	623	612	3	175			9				13	1
Georgia	2		13	989		18			2		1		9	10
Florida	1			210	2	22	2		5			3	1	
EAST SOUTH CENTRAL														
Kentucky	4		2	363	4	29			38	1		2	54	12
Tennessee	2		51	563	4	70			20		1	1	62	
Alabama	4	1	75	333	2	75	2		6			2	4	5
Mississippi [a]	8		14	170	2	28	4		8		2	1	1	
WEST SOUTH CENTRAL														
Arkansas	8		121	712		38	1				5	1	14	1
Louisiana	3		1	87	1	38	2		3			4		1
Oklahoma	2		27	380	2	16	1		5		1	1		7
Texas	29		931	2,405	4	312	7		26	1			116	26
MOUNTAIN														
Montana				153					9				7	
Idaho	1		15	155		7		1	[c] 9				1	
Wyoming				26										
Colorado			13	276		16			8			5	4	
New Mexico	1			180		8			8	1				
Arizona	2		68	242		25			13				19	
Utah [a]				94	1	3	8		5				13	
Nevada												1		
PACIFIC														
Washington			2	576		3			27				4	
Oregon	1		9	279	2	7	2		12			3	11	
California	9	1	12	1,261	6	27	5		[c] 86			2	41	3
Total	164	10	2,288	29,165	78	1,968	45	4	2,076	5	14	50	1,020	
Median, 1944–48	195	8	1,691	27,438	112		32	2	3,833	9	13	68	1,952	
Year to date, 16 weeks	2,659	126	63,323	372,880	1,364	37,603	1,147	22	44,091	32	412	680	15,991	
Median, 1944–48	4,432	131	180,632	278,171	2,949		553	11	56,286	158	291	759	34,858	
	(27th)		(30th)	(35th)	(37th)		(11th)		(32d)	(35th)		(11th)	(39th)	
Seasonal low week ends	July 10		July 31	Sept. 4	Sept. 18		Mar. 19		Aug. 14	Sept. 4		Mar. 19	Oct. 2	
Since seasonal low week	7,773		99,593	425,273	2,208		225		66,789	47		220	26,024	
Median, 1943–48	11,998		311,728	313,117	4,453		142		94,867	234		280	61,295	

[a] Period ended earlier than Saturday.
[b] New York City and Philadelphia only, respectively.
[c] Including cases reported as streptococcal infection and septic sore throat.
[d] Including paratyphoid fever; reported separately, as follows: Virginia 1; North Carolina 1; Florida 2; Louisiana 1; Colorado 1; Oregon 1; California 1; salmonella infections, not included, were reported as follows: New York 1; Pennsylvania 1.

Anthrax: New York 1.
Alaska: Measles 2; scarlet fever 1.
Territory of Hawaii: Diphtheria 1; measles 190; lobar pneumonia 1.

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended April 2, 1949.—During the week ended April 2, 1949, cases of certain communicable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox		24	9	242	601	32	24	43	236	1,211
Diphtheria		2		8		1		6		17
Dysentery, bacillary				10	3					13
German measles				319	64	1	213	16	10	623
Influenza		99			17	23			4	143
Measles		128	124	132	204	135	118	245	268	1,354
Meningitis, meningococcal		1				2				3
Mumps		36	7	102	439	48	25	16	148	821
Poliomyelitis				1		2				3
Scarlet fever		4	1	101	90			11	7	214
Tuberculosis (all forms)		4	13	151	30	11	7		38	254
Typhoid and paratyphoid fever			4	6						10
Venereal diseases:										
Gonorrhea	1	10	4	85	57	27	15	25	63	287
Syphilis	1	5	11	84	50	5	6	6	17	185
Whooping cough		17	1	115	24	4	7			168

GOLD COAST

Cerebrospinal meningitis.—Information dated April 21, 1949, states that 836 new cases of cerebrospinal meningitis, with 59 deaths, were reported in the Northern Territories, Gold Coast, during the period March 27–April 2, 1949. A total of 8,525 cases, 622 deaths has been reported in that area to date in the present outbreak.

NIGERIA

Cerebrospinal meningitis.—According to information dated April 5, 1949, 26,734 cases of cerebrospinal meningitis, with 6,359 deaths, have been reported in the Northern Provinces, Nigeria, from the beginning of the year to April 2, 1949. Confirmed figures reported for the week ended April 2, are as follows: Argungu, 460 cases, 36 deaths; Gusau, 1,063 cases, 109 deaths; Katsina, 975 cases, 83 deaths; Port of Sokoto medical area, 1,650 cases, 383 deaths; Sokoto City. 16 cases, 2 deaths.

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

NOTE.—The following reports include only items of unusual incidence or of special interest and the occurrence of these diseases, except yellow fever, in localities which had not recently reported cases. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Cholera

India—Calcutta.—During the week ended April 9, 1949, 255 cases of cholera with 69 deaths were reported in Calcutta, India.

Plague

India.—During the week ended April 9, 1949, 29 cases of plague with 6 deaths were reported in Cawnpore, India, and 10 cases, 1 death, were reported in Calcutta.

Peru.—For the period March 1–31, 1949, plague was reported in Peru as follows: In Uchupata, Huancabamba Province, Piura Department, 3 cases, 1 death; in Monsefu, Chiclayo Province, Lambayeque Department, 1 case, 1 death.

Smallpox

Egypt—Port Said.—Information dated April 22, 1949, states that an outbreak of nonepidemic smallpox has been reported in Port Said, Egypt. Three cases are stated to have occurred during the previous few days.

Great Britain—England and Wales—Port of London.—According to information received from London, dated April 20, 1949, 7 additional confirmed cases of smallpox have been reported in England, and the number of deaths has risen to 5. All these cases are stated to be considered direct importations from the liner *Mooltan*. One case each has occurred in Torquay, Liverpool, a small town in Lincolnshire, Sutton in Surrey, Richmond, and Aylesbury. The rest are all in the London area. Two of the cases reported confirmed were in persons traveling from London to other points. It is estimated that there may have been as many as 48 contacts altogether.

Portugal—Lisbon.—During the week ended April 2, 1949, 2 cases of smallpox were reported in the city of Lisbon, Portugal.

Republic of the Philippines—Mindoro Island.—Details of the epidemic of smallpox which occurred in Mindoro Island, Republic of the Philippines, during the year 1948, have been received. The first case is stated to have occurred on April 5, 1948, in the town of Pinamalayan, and the last case on December 16, 1948, in the town of Mansalay. The total number of cases reported during this period was

282, with 103 deaths. Distribution was as follows: Pinamalayan 27 cases, 8 deaths; Roxas 14 cases, 5 deaths; Mansalay 178 cases, 66 deaths; Bongabong 63 cases, 24 deaths.

Yellow Fever

Brazil—Amazonas State.—On January 23, 1949, 1 death from yellow fever was reported in Benjamin Constant, Amazonas State, Brazil.

DEATHS DURING WEEK ENDED APR. 16, 1949

[From the Weekly Mortality Index, issued by the National Office of Vital Statistics]

	Week ended Apr. 16, 1949	Corresponding week, 1948
Data for 94 large cities of the United States:		
Total deaths	9,232	9,009
Median for 3 prior years	9,127	
Total deaths, first 15 weeks of year	147,369	151,611
Deaths under 1 year of age	608	659
Median for 3 prior years	659	
Deaths under 1 year of age, first 15 weeks of year	9,915	10,417
Data from industrial insurance companies:		
Policies in force	70,481,914	71,083,995
Number of death claims	10,943	13,429
Death claims per 1,000 policies in force, annual rate	8.1	9.9
Death claims per 1,000 policies, first 15 weeks of year, annual rate	9.7	10.6